# SPECIES CONCEPT AMONG THE ACTINOMYCETES WITH SPECIAL REFERENCE TO THE GENUS STREPTOMYCES

SELMAN A. WAKSMAN

*Institute of Microbiology, Rutgers University, The State University of New Jersey, New Brunswick, N. J.*

## INTRODUCTION

In the study of the taxonomy of any group of living organisms, one is faced sooner or later with the problem of defining what is meant by a species. In usual practice, a certain type of plant or animal, or a microbial culture, is designated by a name; its properties or characteristics are described sufficiently so that anyone else who finds this organism in nature will be able to recognize it from the description. If possible, the type form of the species is preserved as a herbarium specimen and the living culture is preserved in a type culture collection. This may aid greatly in its proper identification in the future.

Unfortunately, biological forms and types of organisms are not fixed in nature or even in culture. Some forms, even those closely related to the fixed type, may vary enough to raise a question of their exact or specific identity. This frequently leads, often on the basis of only minor differences, to the creation of new species that are given new names. This is particularly true of microorganisms, especially those that occur abundantly in nature; some of the newly isolated cultures may vary greatly from the fixed types. The difficulty of establishing and recognizing "species" under these conditions may become particularly perplexing. Raper (51) was fully justified in saying that "It is almost axiomatic that the ease with which a species of microorganism can be recognized tends to vary inversely with the number of isolates available for observation and examination."

## WHAT IS A MICROBIAL SPECIES?

In discussing the nature of bacterial species, R. E. Buchanan (6) emphasized that the "concepts of speciation that may prove very useful in studies of higher forms may be wholly inapplicable to the groups of organisms with which we are at present concerned." Hitchcock (26) considered a species as a unit of classification comprising a coherent group of like individuals. He emphasized, however, that a species is difficult to define with precision because it is not a definite entity, but a taxonomic concept. It is often simply a matter of convenience in classification as to just where the exact line is to be drawn. Hucker and Pederson (29) emphasized that the difficulty of dividing lower forms into well-defined species has led many to question whether these are natural groups and whether they can be considered to be similar to "species" among higher forms of life. The problem always arises, how much difference must exist between two cultures of bacteria before one is justified in regarding them as being distinct species.

Burkholder *et al.* (7) emphasized that the species concepts formulated by individual investigators depend a great deal upon the latter's personal experience, whether he is a "splitter" or a "lumper." They came to the logical conclusion that microbial species (as applied particularly to *Streptomyces*) should be characterized by multiple, readily recognizable, and reasonably stable properties; the history of the cultures and the nature of the medium in which they are growing are of prime importance.

According to Pantin (49), the definition of a species is a "statistical concept" or "a book-making notion." He suggested that there are two distinct methods of recognizing a species: the "field method" and the "analytical deductive method of laboratory systematics." The first must necessarily be based upon "a long series of past experiences," primarily the impression made by the organism; this involves personal judgment. Since bacteria can rarely be seen in nature with the naked eye, and even if seen could hardly be thus recognized as a particular species, one must depend entirely upon "laboratory systematics" or upon the use of a series of well-defined laboratory procedures, which, when taken together, define a species.

In comparing the species concept among microbes with that of higher plants and animals, Cowan (14) suggested the consideration of the following aspects: (a) whereas larger plants and

animals have geographical distribution areas, few microbes have such particular areas; (b) morphology is essential for the separation of species among algae, fungi and protozoa, but it barely distinguishes higher ranks among bacteria; (c) cytology is useful at the generic level, but "at the species level the bacteriologist relies more on physiological than on morphological differences"; (d) interfertility is hardly to be considered as a species character, since bacteria and actinomycetes reproduce asexually; (e) the introduction of certain characters in microbiology not utilized by botanists and zoologists adds satisfactory classification criteria; these include "nutritional requirements, metabolic and catabolic products, antigenic structure and pathogenicity."

Snyder and Hansen (59) have synthesized the concept of speciation among fusaria as follows:

1. Individual cultures are accumulated.

2. An attempt is made to determine, by means of experimental procedures, the limits of variability, especially in respect to morphologic features.

3. This leads finally to the synthesis of the species. This involves the judgement of the investigator, from the analytical data available, as to which characters are common to all individuals of one species, and which serve also to distinguish such individuals from those of other species. Snyder and Hansen added significantly: "When only a few individuals are the subject of a taxonomic study, it is easy to distinguish between them, and these individuals are therefore often named as different species. But when many isolates are assembled it becomes much more difficult to distinguish between the first-named species." A large number of isolates of one species may lead to recognition of "natural groups."

Although time and again taxonomists have emphasized the fact that an effective system of classification should be based upon constant criteria, this can hardly apply, at least as far as our present knowledge is concerned, to the species characterization of bacteria. Many species have been described on the basis of a single characteristic, frequently a quantitative variable. One often wonders what the composition of the medium, the conditions of growth, and the natural variability that one observes so frequently among duplicate cultures, have to do with these so-called distinguishing properties. The examination of a group of cultures freshly isolated from a natural substrate will illustrate this. It is easy to pick out a few cultures that possess distinctive enough properties as to be recognized as distinct species, and discard all the others. Should the various intermediates be considered, however, one might be inclined to consider each as a different species, distinct from the others by at least one variable property, be it cultural, morphological, or biochemical. With the examination, in recent years, of many thousands of cultures of bacteria and actinomycetes for their antibiotic properties, such an attitude could easily be reduced to an absurdity. On the other hand, the arbitrary contention of those who insist upon permanent characteristics, preferably a group of them, would limit greatly our recognition of the growing economic importance of these organisms.

With actinomycetes particularly, the species are so gradually linked together that it is very difficult to say where one species ends and another begins. The creation of "groups" or "series," which occupy an intermediate place between genera and species, helps somewhat in clarifying the relationship, but does not do away entirely with the potential confusion in the creation of new species, when their relation to the species already established is not sufficiently understood.

This confusion had led some investigators to question "whether the species concept is tenable in microbiology, and if it is not, what we are to substitute for it." It was even suggested "that the concept of static species must be abandoned in favor of something more elastic." Also the question is raised: "Can we accept the species concept, and all that this implies, or must we view our organisms as a huge spectrum composed of gradually merging forms?" (14). This is, of course, diametrically opposite to the classical systematics.

### *Species Concept Among Actinomycetes*

What has been said for bacteria and fungi can be applied with special emphasis to actinomycetes. This is true particularly of such a large, heterogeneous, and variable group of organisms as that represented in nature by the genus *Streptomyces*. These organisms are found in the soil in hundreds of thousands of spores and bits of mycelium per gram. They are also found extensively in manures and in composts, in various fresh-water basins, in dust, and on food.

They are almost entirely absent in peat bogs and in the sea.

The actinomycetes have recently come to occupy an eminent place because they are important producers of antibiotics, vitamins, and enzymes. Unfortunately, it is often difficult to establish species differences among actinomycetes and still more difficult to recognize them. This is due to the great variability of these organisms, especially when grown on media of different chemical composition and under different environmental conditions (7, 18, 30, 55). The lack of constancy of certain specific characters among actinomycetes, the ease with which spontaneous mutations are produced, usually in the form of saltations, and the frequent lack of sufficiently recognized type species tend to complicate this problem further. With the growing economic significance of the actinomycetes, especially of the members of the genus *Streptomyces*, the necessity of establishing for each species a group of characteristics which would be sufficient to enable the investigator to recognize freshly isolated cultures in well-defined specific terms becomes of great theoretical and practical importance.

Following the first descriptions of F. Cohn (10) in 1872 (*Streptothrix försteri*) and of Harz (24) in 1877 (*Actinomyces bovis*), very few additional species of actinomycetes were recognized until 1914. In spite of the fact that the rapidly accumulating literature on the occurrence of actinomycetes in the soil and in the causation of plant diseases, notably potato scab, and of certain animal diseases, very few new species were described during those four decades. The common designations were limited largely to "*Actinomyces albus*" and "*Actinomyces chromogenus*," depending on the pigmentation of the mycelium or pigment formation in the substrate.

The limited recognition of species among actinomycetes prior to 1914 was due primarily to the fact that organic media were employed for their cultivation. Most of the species designations gave recognition largely to the source of the culture, color of the aerial mycelium, and formation of soluble pigments. As late as 1921, Lieske (39) mentioned only a few species, in spite of the fact that he worked with many cultures that varied greatly in their physiological and biochemical properties. He did not even believe that the classification of actinomycetes was possible, since the properties observed were highly variable. His skeptical attitude toward the question of speciation of actinomycetes was due to his use of complex organic media for the growth of these organisms, and to a lack of sufficient appreciation of the significance of simple media for their characterization.

With the introduction of synthetic media by Krainsky in 1914 (33) and by Waksman and Curtis in 1915 (67), it became definitely established that we are dealing, in the case of actinomycetes, with a great number of forms, differing greatly in their physiological and biochemical properties, and to a lesser extent in their morphology. The fact was soon established that the actinomycetes comprise a large and heterogeneous group of microorganisms, occurring abundantly in various natural substrates, and possessing a great variety of activities. This was true particularly of the forms that are able to produce aerial mycelium, namely those that were later recognized as members of the genus *Streptomyces*. It was also recognized immediately that, if a sufficiently large number of cultures were isolated and examined, they will be found to show variability in the type species. The concept "species-groups," with one culture as the type species, was suggested. Waksman emphasized, therefore, in spite of variation of individual biochemical properties of the organisms, that there are certain well defined properties that characterize these organisms, especially when grown on standard synthetic media and under carefully controlled conditions of temperature and aeration.

These concepts dominated the literature on the actinomycetes for more than two decades. The belief in the lack of stability of specific characters is illustrated by the work of Henrici, Jensen, and numerous others. On the other hand, Ørskov, Erikson, and others were convinced of the stability of actinomycetes cultures. Hereditary variations were considered largely as mutants. Frequent reference was made to the fact that the dissociation phenomenon observed among actinomycetes largely represented life stages common to members of a particular group. This phenomenon was frequently utilized to emphasize the close relationship between the actinomycetes and the true bacteria.

Prior to 1943, several systems of classification of actinomycetes were proposed. In most in-

stances, all the species were included in a single genus, which was frequently designated by a different name. The most common of these names were the two oldest, *Streptothrix* and *Actinomyces*. Although occasional efforts had been made to separate the actinomycetes into several genera, such attempts usually failed to receive more than passing attention. The work of Ørskov, Jensen, Erikson, and Waksman finally led Waksman and Henrici (68) to suggest, in 1943, the division of the actinomycetes into four genera. A new genus, *Streptomyces*, was created to include those forms that are characterized by the production of a sporulating aerial mycelium. Almost all the important antibiotic-producing organisms were found to belong to this genus.

Unfortunately, this generic separation brought with it a number of new problems, which can be briefly summarized as follows:

1. There is a considerable overlapping among the different genera, notably between certain *Streptomyces* forms that have lost the capacity to produce aerial mycelium and species of *Nocardia*, as brought out in a recent paper by Gordon and Smith (20); there is also overlapping between certain nocardiae and mycobacteria.

2. With the genus *Streptomyces* gaining considerable economic importance, the creation of many new species based upon biochemical properties resulted in much confusion in the recognition of distinct species.

3. The use of various mutagenetic agents, such as irradiation, led to the formation of new forms or strains which are often markedly different in their nutrient requirements and biochemical activities from the original cultures.

4. The formation by *Streptomyces* species of two different types of mycelium, vegetative and aerial, and the influence of previous conditions of cultivation upon the growth and biochemical activities of these organisms served to complicate the existing confusion further.

Even synthetic media did not yield the final answer to the species characterization of this group of organisms. Their cultural properties, or growth characteristics in media of different chemical composition, properties that were at first greatly emphasized, were found to be extremely variable. Type cultures were shown to change their specific characteristics when grown in artificial media. Saltations and mutations came to play a highly important part in changing such properties. When morphology was recognized at all, it was limited largely to observations on the curvature of the aerial mycelium or to the size and shape of the conidia. Although Drechsler (16) made a detailed study of the morphology of some of the actinomycetes producing aerial mycelium, he limited himself to a small number of cultures. This prevented him from establishing the existence of many specific types, which could have been recognized on the basis of not only cultural but also of morphological properties.

The eminent American cryptogamic botanist, Roland Thaxter, who had himself described many years earlier (63) an important economic species, the causative agent of potato scab (which he believed to be a fungus, *Oospora*), was highly critical of the efforts to describe "species" largely on the basis of cultural properties of the organisms. It took a personal visit on the part of the writer (in 1919) and nearly a day's discussion to convince Dr. Thaxter that at that stage of our knowledge of the actinomycetes, the cultural properties of the organisms grown on media of constant chemical composition had to be considered as the major reliable criteria upon which species descriptions could be based. Too little was still known of their morphology to justify using such criteria alone for species characterization.

In this respect, the actinomycetes do not differ from any of the other groups of bacteria, where cultural properties and biochemical reactions have to supplement insufficient morphological information. Unfortunately, physiological activities, which are the expression of the response of organisms to their environment, are too numerous and often too variable as regards actinomycetes to justify unlimited confidence.

Even now, after considerable additional data have accumulated concerning the morphology of the actinomycetes and after they have been separated into four genera, there is still no general agreement concerning characterization of species. Krassilnikov (34, 35) insists that the shape of the spore should be recognized as the major criterion for species differentiation. One may doubt, however, whether Krassilnikov's numerous "longisporus" and "globisporus" types, with their many subtypes, will greatly facilitate the solution of the problem of species characterization. The writer is more convinced now than he was 40 years ago, when he first began his studies of the actinomycetes, that the

cultural properties of these organisms still offer some of the most important criteria for species differentiation. Furthermore, there is now available sufficient additional information concerning their morphology, such as formation and branching of the conidiophores, as well as formation and nature of conidia, to make possible the use of these criteria not only for supplementary but often for major characterization of the species.

These considerations are, of course, true primarily of the genus *Streptomyces*, which is the group that has recently attracted the greatest attention. The wide practical applications of these organisms carry with them the danger of still greater confusion, since many workers find it much easier to give a new name to an economically important culture than to attempt an identification with one that has already been described. The fact that the creation of a new species may facilitate the patent situation serves to aggravate matters. On the other hand, the importance of a particular biochemical product may offer the temptation of grouping all cultures producing such a substance into a single species.

Several factors have thus contributed toward the confusion in establishing and recognizing species of actinomycetes: (a) lack of clearly defined morphological characters; (b) great variability of these organisms; (c) occurrence of numerous transition types; (d) ease of formation of mutants; (e) lack of sufficiently recognizable type species; (f) lack of emphasis upon species-groups and upon type cultures; (g) insufficient knowledge of the formation of well-defined chemical compounds, which could be used as additional criteria for species characterization.

In establishing and defining new species among actinomycetes, we thus find continuous reflections of the old battle between Cohn (10) and Nägeli (45). Cohn and his group insisted that the species that they created were no less constant in their properties than those of higher plants. Nägeli and his adherents, however, denied the existence of constant characteristics among bacterial species; he was inclined to place all bacteria which possess certain variable characteristics into one species. Nägeli considered the various new species of bacteria established by Cohn as mere modifications of the same organism originating under the influence of changing conditions of environment. It was later found that Nägeli worked largely with contaminated cultures. This process of reasoning would no doubt apply also to the more recent proponents of the pleomorphistic, so well illustrated in the "Life cycles of bacteria" by F. Löhnis (41).

Although species of higher forms of life are differentiated primarily on a morphological basis, the speciation of microscopic and submicroscopic forms has to depend necessarily upon their physiology. The preservation of type cultures is a primary requirement; unfortunately, these too undergo marked variation upon continued cultivation. The suggestion that closely related strains be placed into "species-groups" or "aggregate-species" has recently been gaining considerable attention. Such a "species" should be characterized by various reproducible properties under standard conditions of culture.

Baldacci *et al.* (3, 4) suggested that micromorphological criteria, namely segmentation and branching of vegetative mycelium, presence or absence of conidia, and arrangement of conidiophores, be used for generic classification. He also recommended that each genus be divided into groups or "series," on the basis of cultural characteristics of the organisms. Each "series" is to be divided into species, on the basis of enzymatic activity, antibiotic production, and formation of soluble pigments, as influenced by nutrition and reaction. The genus *Streptomyces* was thus divided, on the basis of pigmentation of the vegetative and aerial mycelium, into a number of "series," each of which was further sub-divided into species (see appendix III).

Any variation from the described species, be that a single qualitative property, such as the production of a specific antibiotic, or be it a quantitative difference, such as the degree of liquefaction of gelatin or depth of color of a soluble pigment, may be ascribed to a varietal rather than a species difference. Any variant can thus be also characterized as a strain of a species from which it originated. The concepts and designations of "strain," "type," and "variety" have been frequently confused, when referring to the source of the organism *vis-a-vis* its biological variation.

As has already been mentioned, when so many different cultures of actinomycetes can easily be isolated from natural substrates, it is but natural that various intermediate types should be found, and that established species should tend to overlap one another. If one were to isolate only one or very few cultures, it would be very simple to

recognize one or a few well-defined species. But when hundreds of similar strains are found in nature, when many of them show only minor variations from one another, not important enough to warrant creation of new species, but which are nevertheless variations from the established type, the difficulties mount rapidly.

Based upon the study of a single strain, a particular species may be described as having a yellow or yellowish aerial mycelium. Another strain may produce, on the same medium, an aerial mycelium only a shade different in color from the original type; this pigment may be designated as sulfur-yellow, cream-yellow, saffron-yellow, or even brownish, all other physiological and morphological properties being similar. Would one be justified in calling such a new strain a different species? The answer is definitely "no". One culture may produce a strong tyrosinase reaction, and another only a weak reaction, as indicated by pigmentation on potato, gelatin and other protein media. One would be inclined to accept these as mere quantitative variations allowable for an established species. This must be recognized since it is well known that had the test been repeated in a different laboratory, where the medium might be slightly different in composition, the method of sterilization of the medium different, or the age and origin of the inoculum not the same, these variations might have been sufficient to account for the minor differences in the color of mycelium or in the pigmentation of the medium. But what is one to do when the original culture is recorded as producing a yellow aerial mycelium on a given medium, whereas the new isolate gives a buff or brown or gray mycelium? The answer would be that if all the other recognizable properties are the same or similar, this would be nothing more than a variant. Were one to plate out a single culture and pick a large number of colonies, similar variations could no doubt be observed.

Unfortunately, it has frequently been found much easier to assign undue importance to these variations and designate a freshly isolated culture as a new species. Some justification in this attitude has been found in the fact that the new culture possesses an important economic property, as that of producing a new antibiotic; this was frequently correlated with certain other morphological or cultural differences. This is largely the reason why within the last 10 years there have been more "new" species created than in all the previous 75 years since Ferdinand Cohn first described his *Streptothrix försteri*.

## SYSTEMATIC POSITION OF ACTINOMYCETES

The taxonomic position of the actinomycetes, notably their relationship to the bacteria, on the one hand, and to the fungi, on the other, has been one of the most debatable questions in microbiology. Their size (width of thallus) and their staining properties have usually placed them with the bacteria (6, 21, 38, 71). Their branching and manner of sporulation suggested their relationship to the fungi (8*a*, *b*, 49, 63). Still other properties of actinomycetes seemed to warrant their consideration as a transition group between the bacteria and the fungi (22, 34, 35, 38, 39, 64, 65).

Recent evidence seems to point definitely to the fact that the actinomycetes are not to be considered as higher fungi, since they appear to be more closely related to the bacteria. This evidence can be summarized briefly as follows:

1. Some of the actinomycetes, notably species of *Actinomyces* and *Nocardia* are closely related to some bacteria, notably *Lactobacillus* and *Corynebacterium*.

2. Neither actinomycetes nor bacteria have been shown to contain true nuclei; they both contain only chromatin granules distributed through the hyphae or the cells (23).

3. The diameter of actinomyces mycelium and spores is similar to that of bacteria. Actinomycetes also lack, as a rule, septa.

4. Actinomycetes are subject to attack by phages just as bacteria are; true fungi are not.

5. Actinomycetes are usually sensitive (allowing for strain variability) to antibiotics that are active upon bacteria; they are usually resistant to those antibiotics that are active upon fungi but not upon bacteria.

6. Chitin is absent in the cell substance of actinomycetes as well as in bacterial cells, but is present in fungus mycelium and spores. In their lack of cellulose, actinomycetes are also similar to most bacteria rather than to fungi. Avery and Blank (2) concluded that "from the chemical point of view *Actinomycetales* have nothing in common with the true fungi, but rather with the bacteria."

7. Like bacteria, but unlike most fungi, actinomycetes as a rule are sensitive to acids.

8. The close relationship of the actinomycetes

to the bacteria is also evident from the work of Couch (11), who found that certain *Micromonospora*-like forms resemble those of bacteria. He suggested the creation of a new family, *Actinoplanaceae*, with two new genera *Actinoplanes* and *Streptosporangium* (12, 13). Couch emphasized the resemblance of the mycelium and sporangia of *Actinoplanes* to those of the chytrids; he concluded that this genus may represent a connecting link between the bacteria and the lower fungi.

Further discussion of speciation among actinomycetes will be largely limited to the genus *Streptomyces*.

## CHARACTERIZATION OF A STREPTOMYCES SPECIES

The existence of physiologic races or varieties in any living species has been fully recognized. This is true particularly of species of actinomycetes. Just as in improving higher forms of life, one is always faced with the selection of varieties resistant to disease, or giving higher yields, or having other desirable qualities, so one must select strains of actinomycetes on the basis of resistance to phage, or of production of higher yields of a given antibiotic or other metabolic product. It becomes particularly important to establish type species or group-species, within which a certain degree of variation may be permitted, before making an attempt to establish new species.

To characterize a species of an actinomycete, especially a member of the genus *Streptomyces*, certain morphological and cultural properties should be considered. These are based upon the growth of the organism on a variety of different media, including complex plant and animal products, such as potato, milk, and gelatin, and artificial media, comprising both organic and inorganic or synthetic media.

### *Morphological Properties on Selected Media*

As in any group of living systems, members of the genus *Streptomyces* are characterized by certain distinct morphological properties which can be used if not as major, at least as minor, criteria for their specific differentiation. The nature of the medium has an important bearing upon the morphology of the organism. Colony formation, vegetative mycelium, aerial mycelium, and spores are most important in this connection.

*Colony.* An actinomycete colony is not a colony in a true sense, as the term is applied, for example, to bacteria. It is rather a mass of mycelium that originates from one spore or from one piece of mycelium, or is a filamentous extension of the parent cell. The colony of a *Streptomyces* is composed of a vegetative or substrate mycelium and of an aerial or conidia-forming mycelium. There are marked differences in the growth characteristics of both types of mycelium. The kind of inoculum, especially vegetative *vis-a-vis* spore inoculum, has an important effect upon the growth of the culture.

The nature of the actinomycete colony growing on a standard agar plate has been considered as among important criteria for characterizing and recognizing a particular organism. The morphology of the colony, notably its general appearance, size, shape, and texture; the formation and pigmentation of the aerial mycelium; the production of soluble pigments in the medium—can all be readily determined by superficial examination. Various other properties may be recognized from a study of the colony.

Although Krainsky (33) and others used the structure of the colony, especially its size and shape, as one of the major diagnostic criteria, one must question, however, its significance. The other properties are also important in describing species.

*Formation and nature of vegetative mycelium.* Vegetative mycelium, or substratum mycelium, as it is frequently referred to, has the growth properties characteristic of the genus *Streptomyces*. It does not segment spontaneously into bacillary or coccoid forms. The mycelium produces the leathery, or tough-textured growth, remaining non-septate and coherent even in old cultures. Although no true septa are observed in young cultures, it has recently been claimed that older cultures at least show occasional septation. The compactness of this vegetative growth is responsible for the fact that liquid media are always clear, unless the culture has been subject to phage or lytic action.

Vegetative growths of *Streptomyces* produce soluble and insoluble pigments; this property contributes to their specific differentiation. Some strains may lose the capacity to form aerial mycelium under certain conditions; such cultures may frequently be mistaken for nocardias.

*Formation and nature of aerial mycelium.* The aerial mycelium is usually thicker than the vegetative mycelium. Several aspects relating to the aerial mycelium may be considered:

a. Gross macroscopic appearance of the mycelium in cultures. The relative abundance of aerial mycelium, its structure (cottony, velvety, powdery), the formation of rings or concentric zones of alternating sporogenous and vegetative phases, and the pigmentation of the mycelium are important diagnostic criteria.

b. Microscopic properties. The use of morphological criteria is a significant, if not a leading, factor in establishing a sound taxonomic basis for the actinomycetes. This was recognized by Thaxter (63) and Drechsler (16), later by Krassilnikov (34, 35) and Waksman *et al.* (68, 69). The microscopic structure of the aerial mycelium gives a clear picture of the morphology and reproductive properties of the organism. The hyphae may be long or short, with extensive or little branching. The latter may be simple or complex, monopodial or sympodial, broom-shaped or verticillate. The conidiophores are short or long, occurring singly, in clusters, or as whorls; they are straight, wavy, or spiral-forming. The spirals are either long and open or short and compact. Spiral formation may take place on one medium only. Before a culture is pronounced as not forming any spirals, however, it must be grown on a variety of different media. Drechsler suggested the use of the right-hand or left-hand curvature of the spirals as a diagnostic feature, but this, too, is influenced by the composition of the medium. Whorl formation is a much more important characteristic of the species; it can be simple or branching, the branches being straight or forming spirals; but this property as well is influenced to some extent by the composition of the medium. Although nocardias may produce sporulating aerial filaments, these are never spiral-shaped.

Burkholder *et al.* (7) illustrated the micromorphology of several species-groups of *Streptomyces* as follows: *S. albus*—Long, spore chains spiral. *S. antibioticus*—Straight, arranged in clusters or broomlike. *S. flavus*—Long, spiral-shaped. *S. griseus*—Straight or flexuous, often in tufts. *S. lavendulae*—Branches not flexuous, often forming loops and loose spirals. *S. reticuli*—Verticillate, spore chains spiral or straight.

c. Conidia. These may be oblong, oval, or spherical. Krassilnikov (34, 35) attached great importance to this character as a diagnostic feature. Küster (37) classified the conidia into: 1. Those producing a smooth surface; 2. Those having a rough surface. Each of these groups was subdivided into three subgroups, based on shape of conidia. When cultures are grown on optimum sporulation media, the pigmentation of the conidia is highly significant. It may be observed at an early growth stage, at maturity, or only in old cultures, since changes in color may occur with age of culture.

On the basis of a system of classification that they have outlined, Baldacci and Grein (4) examined 50 strains of actinomycetes by the electron microscope. Three types of conidia were recognized: 1. Oval, more or less transparent conidia; these were either smooth or rough, the latter having a spiny or hairy surface; the spines were either short and thick or long and thin. 2. Round, opaque conidia, usually smooth. 3. Polyhedral conidia, smooth and transparent, or slightly curved, wrinkled and opaque. The form of the conidium was constant for the series, but could not be used as a species characteristic.

*Staining reactions.* The gram-stain and acid-fast stain are of little diagnostic value in separating species of *Streptomyces*. A variety of stains are used, however, for the examination of the structure of sporulating hyphae and of the conidia.

### *Cultural and Biochemical Properties*

*Formation of pigments.* Among the cultural properties of actinomycetes, the formation of soluble and insoluble pigments, both in the vegetative and in the aerial mycelium and in the conidia, has come to play a major role in characterizing species. This is demonstrated by the use of numerous specific names, based upon the nature of the pigments, in describing various organisms. Unfortunately, this property varies greatly with age of the culture, composition of the medium, temperature of incubation, and nature of the inoculum.

Before the introduction of synthetic media, and even more recently, it has been a common practice to divide the actinomycetes into: (a) colorless forms, and (b) pigment-producing types. But with the introduction of synthetic media, it came to be recognized that different organisms produce a great variety of pigments, ranging from red to blue, and from orange and yellow to brown and black. Some are single pigments and others comprise two or more constituent pigments. Some are water-soluble (chromopar) and others are water-insoluble (chromophor). The presence of oxygen is essential for pigment forma-

tion. The pH of the medium greatly affects the nature of the pigment, both in the insoluble and soluble forms.

The formation of brown to black pigments on organic media containing proteins and protein derivatives (tyrosine) is an important species characteristic. It was the ability to produce this type of pigment that led many of the earlier investigators to designate certain species as "chromogenic." Intermediary strains may produce only faint brown pigments. Different cultures, especially on continued cultivation on artificial media, will show great variation in pigment production. On synthetic media, the formation of yellow, red, blue, green, and other soluble pigments is also highly characteristic of the species. There is considerable variation in the intensity of the pigment, depending upon the strain of organism, variation in composition of the medium, and prior cultivation.

In view of the fact that color standards are not always available, Lindenbein (40) suggested a series of color designations which are simple and convenient. This system in a modified form is given in Appendix I.

*Utilization of carbon sources.* The ability of different species of actinomycetes to utilize various organic compounds, such as carbohydrates, alcohols, salts of organic acids, fats, and amino compounds, as sources of carbon is of considerable diagnostic value. Production of specific enzymes, including diastase, invertase, inulase, lipase, and steroid-oxidizing systems, is also of importance. Here again, there is considerable variation in the behavior of different isolates belonging to the same species or closely related species; test conditions, and origin and age of culture are also of importance.

*Utilization of nitrogen compounds.* Availability of different nitrogen compounds for the growth of actinomycetes may offer criteria of certain diagnostic value. Certain chemical changes, such as proteolytic activity, nitrate reduction, and ammonia formation, as well as pigmentation (tyrosinase reaction), supply additional information for species differentiation.

Among the proteolytic activities which are of diagnostic value in separating genera, liquefaction of gelatin and peptonization of milk are important. The *Nocardia* group shows little if any liquefaction of gelatin, whereas most of the species of *Streptomyces* show positive liquefaction. The degree of rapidity of liquefaction varies greatly. Some species show strong activity and others give only limited liquefaction. This property, when combined with the ability of the species to produce brown to black pigments, provides significant criteria.

*Production of specific chemical compounds.* Actinomycetes, especially species of *Streptomyces*, have been found in recent years to produce a series of highly valuable chemical substances. These include antibiotics, vitamins, and enzymes.[1]

The fact that a large proportion of all the cultures of *Streptomyces* that may be isolated from natural substrates show some degree of inhibition of growth of other microorganisms, when tested on suitable media (36, 69), suggests that the production of antibiotics may be of potential diagnostic value. It must be emphasized, however, that the ability to produce a particular antibiotic is a strain rather than a species characteristic. Certain antagonistic strains of *S. griseus*, for example, are able to produce streptomycin; others may form grisein, streptocin, or candicidin.

In making the test, the culture is streaked on suitable agar media and allowed to incubate for 2 to 3 days. The plates are then cross-streaked with a selected group of bacteria, fungi, or actinomycetes, including strains made resistant to various antibiotics. The zones of inhibition measuring the antibacterial and antifungal activities of the particular culture may be used as supplemental criteria. The fact that the growth of homologous strains of an organism is less inhibited than that of heterologous forms adds further weight to the potential diagnostic value of this reaction. The inability of an antibiotic-producing organism to grow in the presence of the particular antibiotic is known as reciprocal antibiosis. The application of this concept to the speciation of actinomycetes is not generally accepted, however, since the metabolism of these organisms is too complicated to give sharp lines of autoinhibition.

*Phage effect.* Stocker (61) has shown that

[1] The ability of *Streptomyces* species to produce diastatic, proteolytic and oxidative enzymes is now well established. A diastatic preparation, biolase, has been introduced into practical use by certain Russian investigators. Noval and Nickerson recently reported (46) on the ability of certain *Streptomyces* cultures to produce keratinolytic enzymes.

phages can find a place as taxonomic agents for bacterial classification. Just as with phage-sensitive bacteria, a particular phage will bring about the lysis of only specific actinomycete strains. The sensitivity of two strains to a single phage suggests their relationship to one another. The formation of phage-sensitive and phage-resistant strains among certain actinomycete species may be accompanied by changes in morphology of the organism. Phages of actinomycetes also vary in their specificity, some attacking only a single strain and others acting even upon strains belonging to different species, as shown by Carvajal (9).

Highly potent streptomycin-producing cultures often lose a great deal of their vitality, such as ability to grow on simple media, production of aerial mycelium, and sporulation. The ability of strains of *S. griseus* to form streptomycin appears to be correlated with the sensitivity of the organism to this antibiotic, as well as with various other biochemical activities, such as rate of glucose utilization, change of pH of the medium, and lysis.

### *Serum Reactions*

It has been suggested to utilize immunological techniques, notably those of agglutination and precipitation, for species identification of actinomycetes. Aoki (1) was thus able to differentiate between representatives of three of the genera of actinomycetes, *Actinomyces*, *Nocardia*, and *Streptomyces*. By the use of sonic vibrations, Ludwig and Hutchinson (42) prepared antigen suspensions satisfactory for use in agglutinin and precipitin reactions and for the production of immune sera in rabbits. Their use in the identification of actinomycetes was suggested by Yokoyama and Hata (73), who reported that a purified antigen of a streptomycin-producing strain was active against immune sera of the same strain, but not against sera of other antibiotic-producing organisms; they were thus able also to establish the close relationship of luteomycin and chloramphenicol-producing organisms.

Ochoa and Hoyos (47) found a correlation between microscopic morphology and serological reactions, which made it possible to divide the actinomycetes into 4 groups, the first comprising species of *Actinomyces* and *Nocardia*, the second largely *Nocardia*, and the third and fourth comprising species of *Streptomyces*. Slack *et al.* (58), however, found that antisera prepared with *A. bovis* brought about low titer agglutination of *Nocardia* sp. and of two species of *Streptomyces*. They concluded that a close antigenic relationship exists between members of the genus *Actinomyces* and a group relationship between *Actinomyces*, *Nocardia* and *Streptomyces*. Much more work on the serological relationships is needed before their significance can be properly evaluated.

### *Chemical Composition*

The occurrence of specific chemical compounds in the cells of the organisms suggests possible differentiation between groups of actinomycetes. This is true, for example, of the occurrence of diaminopimelic acid, a property that may prove to be of generic rather than of specific significance (72). Romano and Sohler (52) have shown that cell walls of *Streptomyces* can be solubilized by lysozyme, suggesting the presence of a mucopolysaccharide; on the other hand, cells of *Nocardia* do not possess this property.

### *Ecological Conditions*

These include the specific occurrence of different organisms in natural substrates. Various attempts have been made to utilize the ecology of the organisms as a basis of classification. These include the effect of temperature, reaction of substrates and causation of disease.

The temperature at which an organism is grown greatly affects the nature and amount of growth, the nature and extent of sporulation, and the formation of soluble pigments. The optimum temperature for the growth of most species of *Streptomyces* is between 25 and 30 C. Only a few of these organisms are thermophilic (66). The division of species into mesophilic and thermophilic has been recognized as an important criterion in establishing species.

The optimum reaction for the growth of actinomycetes is pH 6.8 to pH 7.5. When grown on complex organic media, and even on many of the synthetic media, the reaction usually becomes alkaline. Some actinomycetes are able to grow, however, at pH 4.5 to 6.5 and even at pH 3.0 to 4.5. Such species are not common, but the reaction of the substrate has been recognized as a potential diagnostic property.

On the basis of their pathogenicity actinomycetes have been divided into saprophytic and

parasitic, the latter being further subdivided into plant and animal parasites. Thus we speak of actinomycosis, caused by *A. bovis*, and nocardiosis, caused by different species of *Nocardia*. We associate *S. scabies* with the disease of potato tubers and *S. ipomoea* with a disease of sweet potato roots.

Many other criteria have been utilized for recognizing species of actinomycetes, although usually they are varietal rather than species characteristics. It is sufficient to mention the chemical composition and genetic relationships.

### *Genetic Relationships*

Still little is known about the genetic properties of actinomycetes, but certain suggestions have been made recently which offer rather promising leads. The concept of vegetative hybridization of *Streptomyces* cultures has recently been suggested (28). By repeated growth of a culture in a sterile filtrate of sand-macerated mycelium of another culture, the former underwent morphological and physiological changes. The significance of this phenomenon and its potential utilization for species characterization are still to be elucidated. Sermonti (56) also demonstrated recently several types of recombination among "wild" and mutant strains of *Streptomyces coelicolor*.

## DESCRIPTION OF SPECIES

### *Systems of Classification*

To describe a species of an actinomyces, it is desirable to use as many of the morphological, cultural, and other properties as possible. Only then is there hope that the species or one of its varieties may be recognized. It is essential to use standard media, although each investigator may have a preference as to particular media (Appendix II). In addition to those suggested here, certain other media which appear to yield additional differentiating properties may also be employed, as will be discussed later.

Three simple systems of classification of *Streptomyces* based largely upon these criteria, as well as a system of separation of the genus into "series" are presented in Appendix III.

### *Type Cultures*

One of the important aspects of species characterization consists in a comparison of freshly isolated cultures with type cultures. For the higher forms of life, this has been possible through the use of herbarium specimens. For microorganisms, special collections are available for the purpose of overcoming such difficulties as microscopic forms of life represent.

The need for establishing type cultures of actinomycetes would be indispensable were it not for the unfortunate fact that such cultures undergo considerable variation when grown for a long time upon artificial culture media. Many of the cultures lose their ability to produce aerial mycelium and are thus deprived of a group of properties of major diagnostic value. Unknown cultures of *Streptomyces* free from aerial mycelium may even be considered as species of *Nocardia*.

The prior growth of the culture on soil media (sterile soil treated with a small amount of $CaCO_3$, if acid, and with a half per cent of dried blood), or in carbon, nitrogen, poor media (40), its refrigeration (27), or its lyophilization—each or all tend to overcome the problem of degeneration and thus preserve the original characteristics of the type culture. The cultures that have already degenerated will tend to regain their original properties as a result of such treatments.

### *Variability of Streptomyces Species*

*Streptomyces* species are highly variable. Minor changes in the composition of the medium or in environmental conditions will bring about a marked change in growth and biochemical activities. Among the most variable properties, one may include the pigmentation of the colonies and formation of soluble pigments, the production of aerial mycelium, susceptibility of the culture to phage and lysis, and formation of specific antibiotics. The anaerobic growth of certain actinomycetes is also subject to variation, since they tend to become aerobic upon continued cultivation. The thermophilic forms are also subject to marked change, especially when the cultures are adapted to grow under mesophilic conditions.

A study of a number of strains of *S. griseus* demonstrated that this organism is subject to the following variations (70).

1. Morphological variations when grown on different media.

2. Nature and pigmentation of the aerial mycelium when grown not only on different media but even on different lots of the same medium.

3. Pigmentation of the vegetative growth and formation of soluble pigments.

4. Physiological or cultural properties of different strains of this organism.

5. Utilization of different nutrients.

6. Formation of enzyme systems, notably protease and diastase.

7. Antibiotic production, some strains forming the antibacterial antibiotic streptomycin and the antifungal antibiotic cycloheximide, others producing candicidin or grisein, and still others yielding other antibiotics or none at all.

8. Phage sensitivity and the development of phage-resistant strains.

9. Formation of strains highly potent as regards the production of a particular chemical substance.

*Types of variation.* The foregoing variations are quantitative or qualitative, or both; they are either constant or transient. A change in the makeup of the morphological and cultural properties of an organism, resulting from one or more variations, is sufficient to suggest the potential formation of a new variety. In the streptomycin-producing culture of *S. griseus*, several mutants were obtained, under special conditions of cultivation. One of these has lost the property of forming aerial mycelium and another gained the capacity of pigmentation of its vegetative growth. These changes were accompanied by corresponding changes in antibiotic production, the first losing its capacity to produce streptomycin and the second gaining the property of forming a totally different substance, rhodomycetin. Whether the mycelium-producing property is related to these changes in antibiotic production is a matter of great physiological and practical importance. Not all strains, however, which have lost the ability to form aerial mycelium have also lost the capacity to produce streptomycin (17).

*Limits of variation.* It is difficult to establish the limits of variations of certain properties of *Streptomyces* cultures, especially since such variations are usually quantitative rather than qualitative in nature. Where a property is important, however, a large number of replicates should be prepared, and the observations reported on a statistical basis. It may be necessary to repeat such tests under certain variable conditions, such as the nature of the inoculum, temperature of incubation, and other important facts that influence such variations, so as to make certain that the variability factor has been reduced to a minimum.

### *Standard Media*

Some media are more favorable to sporulation of *Streptomyces* cultures than others. In view of the importance of sporulation in characterizing a species, it is essential to select such media. Further, since some cultures tend to lose the property of sporulation on continued growth, special precautions must be taken in examining such cultures. The loss of aerial mycelium may be reversible or irreversible. Since a nonsporulating culture of *Streptomyces* may resemble a *Nocardia*, and since certain nocardias tend to produce aerial mycelium, the element of confusion between species belonging to the two genera always exists.

The following media* are recommended for the study of actinomycetes:

*Synthetic media*†

| *Essential media* | *Supplementary media* |
|---|---|
| Czapek's agar | Czapek's solution‡ |
| Glucose - asparagine agar | Glycerol-asparagine solution |
| Glycerol - asparagine agar | Cellulose solution |
| Ca-malate agar | Tyrosine agar |
| Starch agar | Glycerol agar |

*Organic media*

| | |
|---|---|
| Oatmeal agar | Nutrient-glucose agar |
| Potato-glucose agar | Nutrient-starch agar |
| Yeast-glucose agar | Glucose-peptone broth |
| Nutrient agar | Potato-glycerol agar |
| Yeast-peptone agar | Peptone gelatin |
| Egg-albumen agar | Loeffler's serum |
| Potato plug | Blood agar |
| Carrot plug | Dorset's egg medium |
| Plain gelatin | |
| Litmus milk | |

## DESCRIPTION OF CERTAIN STREPTOMYCES SPECIES

It is commonly believed that to characterize a species on the basis of certain morphological and physiological properties, it is desirable to describe a large number of such properties. This procedure is not always followed, as illustrated by the fact that many new species have been described

* The composition of each of these media is given in Appendix II.

† These may also contain naturally produced pure chemical substances.

‡ Liquid media in general are of questionable value for diagnostic purposes.

recently, largely because it is easier to create a new species than to attempt to correlate the characteristics of a freshly isolated culture with those of known species already described in the literature. This problem has become particularly acute when Company A, for example, presents claims that to produce the same antibiotic or vitamin it is using a different species than that claimed in the patent granted to Company B. This is done, of course, to avoid patent infringements.

It may be of interest to present here, in a modified form, the suggestion of Hesseltine *et al.* (25) concerning the steps to be taken in the taxonomic study of a *Streptomyces*: species or strain or culture.

1. Collection of strains on the basis of pigmentation of aerial mycelium.
2. Study of morphology of strains growing on a number of substrates favorable to sporulation.
3. Examination of color of conidia of strains growing on optimum sporulating media. Five color groups were recognized: (a) lavender, red, or pink; (b) blue, blue-green, or green; (c) yellow; (d) white; (e) gray, gray-brown, olive gray, or dark gray.
4. Study of cultural characters of strains on various synthetic and organic media.
5. An analysis of certain physiological and biochemical properties, notably action on gelatin, starch, milk, and peptone-iron agar, nitrate reduction, utilization of carbon and nitrogen compounds, and antibiotic action.
6. Identification of new strains with known species and preservation of cultures.

### *Analysis of the History and Present Status of Certain Species of Streptomyces*

Several illustrations may be presented here. One might as well begin with an examination of some of the easily recognized species; this will be followed by an analysis of some of the recently created species. The ease and convenience of creating a new species on the basis of information that was not available to earlier workers facilitates the description of the culture and makes the creation of new species rather tempting. Also, as has been mentioned, when a fresh culture is found to be of considerable economic or biochemical importance, even minor differences are often used in the creation of new species or at least new varieties.

*Streptomyces griseus.* A culture of an organism belonging to the group of actinomycetes now included under the genus *Streptomyces* was isolated from the soil in 1914 by Krainsky (33), in Russia, and described as *Actinomyces griseus*. Its vegetative growth on artificial media was colorless; only a small amount of yellowish soluble pigment was produced. The aerial mycelium was of a characteristic green-gray color on both organic and synthetic media. When the concentration of nitrogen in the medium was increased to one-half per cent, the aerial mycelium became white. The culture was only weakly proteolytic.

Soon afterward, in 1915, Waksman and Curtis (67) isolated from various American soils several cultures of what appeared to be the same organism, the comparison being based primarily on the color of the aerial mycelium (64), as published in Krainsky's description. Since this work was done during the years of World War I, Krainsky's original strain could not be obtained for comparative studies. Further investigations by the writer (64), using the 1915 Waksman and Curtis culture and subsequent isolates made by him, tended to cast doubt on the identity of the Russian and American isolates. In his 1919 description of the organism, the writer suggested that this identification be corrected (64). The differences between the two cultures, based on their published descriptions are brought out in table 1.

In spite of these differences, the writer hesitated to change the name of the American culture. This hesitation was due partly to the fact that the organism was found to undergo considerable variation upon continued cultivation on artificial media. The substrate, the temperature of incubation, the length of the incubation period, the amount and nature of inoculum, all tended to exert an influence upon the morphological and cultural characteristics of the organism. At one time milk was clotted at 37C in two days and then peptonized; at another time, under the same conditions, clotting of the milk required 5 to 6 days; at still another time, the milk in some tubes was not clotted at all but was rapidly peptonized. There were other recognizable changes or variations.

Krainsky reported that the aerial mycelium of his culture formed oval conidia, without reference to the nature of the mycelium itself. Waksman and Curtis observed that their isolates produced a mycelium which consisted of long filaments with

little branching. Drechsler (16), studying the morphology of the latter culture, found that the aerial mycelium showed proliferation of fertile branches at moderately close intervals along the axial hyphae, thus suggesting tuft formation, a phenomenon which alone would definitely suggest that the culture should have been identified as a species distinct from that of Krainsky.

TABLE 1

*Characterization of Streptomyces griseus by different investigators*

| | Krainsky (33) | Waksman and Curtis (67) | Bergey's 6th Edition (5) | Krassilnikov (34, 36)* |
|---|---|---|---|---|
| Morphology | | | | |
| Sporophores | | Long filaments with little branching | Produced in tufts | Spiral-shaped, with 2 to 3 compact turns, in the form of bunches of grapes or tufts |
| Conidia | Oval, 1.2$\mu$ in length | Rod-shaped to cylindrical, 0.8 to 1.5 x 0.8$\mu$ | Spherical to oval, 0.8 to 1.7 x 0.8$\mu$ | Spherical to slightly oval, 0.8 to 0.9$\mu$ |
| Czapek's agar | | | | |
| Vegetative growth | | Colorless, turning olive buff | Thin, spreading, colorless, turning olive-buff | Colorless growth on synthetic media |
| Aerial mycelium | | Abundant, powdery, of a water green color. Medium not colored | Thick, powdery, water green color | Well developed, cottony or velvety. Light colored, turning dark to gray. No soluble pigment |
| Calcium malate-$NH_4Cl$ agar | | | | |
| Vegetative growth | Colonies large, well formed, colorless | Thin, greenish yellow with dark shade | | Colorless growth on synthetic media |
| Aerial mycelium | Green-gray; center frequently white; periphery free from aerial mycelium | Water-green color covering the whole growth, except narrow periphery | | Well developed, cottony or velvety. Light colored, turning dark to gray. No soluble pigment |
| Nutrient agar | | | | |
| Vegetative growth | Colorless | Cream-colored | Abundant, cream-colored | Lichenoid. Gray. |
| Aerial mycelium | None, or appears later and is chalk white | Abundant, typical water-green color | Powdery, white to light gray. No soluble pigment | |
| Gelatin | | | | |
| Vegetative growth | Grayish | Greenish yellow or cream-colored | Greenish yellow or cream-colored with brownish tinge | |
| Aerial mycelium | Present | White-gray, with greenish tinge | | |
| Liquefaction | Weak | Liquefaction rapid, without pigmentation | Liquefaction rapid | Most strains liquefy gelatin rapidly |

* It is uncertain whether Krassilnikov's description is based upon Krainsky's original culture or his own 25 isolates of what he considered to be Krainsky's organism.

TABLE 1—*Continued*

| | Krainsky (33) | Waksman and Curtis (67) | Bergey's 6th Edition (5) | Krassilnikov (34, 36)* |
|---|---|---|---|---|
| Potato plug | | | | |
| Vegetative growth | Grayish | Wrinkled, yellowish | Wrinkled, yellowish to brownish | Colorless growth. Plug may be colored black by some strains |
| Aerial mycelium | White gray | Powdery, water-green color | Powdery white | |
| Color of plug | | Brownish | | |
| Glucose broth | | | | |
| Vegetative growth | Submerged flaky; surface colonies appear late and few | Abundant surface growth | Pellicle abundant, much folded, yellowish with greenish tinge | |
| Aerial mycelium | | White to tea-green color | | |
| Milk | Cream-colored ring on surface. Rapid coagulation and rapid peptonization. Sometimes milk hydrolyzed without previous coagulation | | Cream-colored ring. Milk coagulated and rapidly peptonized | Rapidly peptonized; coagulation none or weak |
| Nitrate reduction | Strong | Fair in presence of starch; little or none in presence of sucrose or glycerol | Positive | Limited |
| Production of enzymes and antibiotics | Weak proteolysis of gelatin, strong hydrolysis of calcium caseinate; no invertase; hydrolyzes esculin; medium diastase producer; grows well on cellulose | Strong proteolysis of gelatin, casein and fibrin; no invertase; strong diastase; scant growth on cellulose | Strongly proteolytic and diastatic. Strongly antagonistic and abundant antibiotic producer | Strongly proteolytic and diastatic. No invertase. Growth on cellulose depends on strain. Antagonistic |

The American isolate was used as the basis for the description of *Actinomyces griseus* in all the early editions (1923–1942) of *Bergey's Manual* (5), and served as the type culture of this species in many of the world's culture collections. Credit was always given, however, to Krainsky for having first used that specific name.

In August, 1943, a culture was isolated in the writer's laboratories which produced a highly important antibiotic designated as streptomycin (54). Upon careful examination, this culture was found to be identical with the *Actinomyces griseus* described by Waksman and Curtis in 1915. Since, in the meantime, Waksman and Henrici (68) had proposed that the generic name for the sporulating forms of actinomycetes be changed from *Actinomyces* to *Streptomyces*, the organism became automatically *Streptomyces griseus*. This name has been universally recognized, since 1943, as the official name for the streptomycin-producing organism, and was so designated in the numerous other collections throughout the world: a detailed study of it was made in 1948 (70).

Recently, Krassilnikov (35, 36) came to the conclusion that the Waksman and Curtis culture was different from that of Krainsky. He may have

had an opportunity to compare Krainsky's original isolate with the streptomycin-producing organism, although he did not mention this fact. Since he spoke only of the culture that he himself isolated, from various soils, the impression is left that his own description was based upon these fresh isolates, and not upon a detailed study of Krainsky's original culture. Krassilnikov suggested that the name of the streptomycin-producing organism be changed from *Streptomyces griseus* to *Actinomyces globisporus streptomycini*. This suggested change is most unfortunate for two obvious reasons: first, a well-described specific name is set aside merely for the sake of priority of a name of a culture which very few investigators have ever seen and which does not appear to be available in any culture collection; second, a name of an organism that has become recognized throughout the world because of its important physiological and biochemical properties, and especially because of its capacity to produce a highly important chemical substance, streptomycin, is changed to a trinomial for an insufficiently described variety of an unknown culture.

The results presented in table 1 tend to emphasize further that *Streptomyces griseus*, originally described by Waksman and Curtis in 1916, a description later supplemented by Waksman (64) and by Waksman, Reilly, and Harris (70), and incorporated in all the editions of *Bergey's Manual*, is different from that of Krainsky, based only upon the published description of the latter's culture. The major differences between the Waksman and Curtis culture and that of Krainsky are: (a) tuft formation in the aerial mycelium of the former, as first indicated by Drechsler, who studied it in 1919; (b) the water-green color of the aerial mycelium; (c) the strong proteolytic properties.

An examination of Krassilnikov's description of *Actinomyces griseus* leads one to the conclusion that his own culture is closer to that of Krainsky than to that of Waksman and Curtis. It is believed, therefore, that Krassilnikov had absolutely no justification for changing the name of the streptomycin-producing organism; and it is suggested that his designation of *Actinomyces globisporus streptomycini* be considered as a synonym for *Streptomyces griseus* (Krainsky emend. Waksman and Curtis) Waksman and Henrici.

For the sake of completeness of characterization of this important organism, it is necessary to emphasize the quantitative and qualitative variations of the numerous isolates and of variants obtained. Particular emphasis is laid upon those variations that have a bearing upon the production of antibiotic substances (70):

1. Some of the naturally occurring strains of *S. griseus* are found not to produce any antibiotic at all, at least not under the particular test conditions.

2. A few of the naturally occurring strains of *S. griseus* produce the antibiotic streptomycin and the antifungal antibiotic cycloheximide.

3. The ability of certain strains to form streptomycin can be greatly increased by strain selection and irradiation.

4. Some of the strains of *S. griseus* form grisein as the major antibiotic.

5. Some of the strains of *S. griseus* form only an antifungal antibiotic, candicidin.

6. The streptomycin-producing strains give rise to at least two types of variants or mutants, one of which loses the capacity to produce aerial mycelium and the other forms a pink to red vegetative mycelium and yields rhodomycetin, an antibiotic that is distinct from streptomycin.

7. Some investigators have recognized five variants of *S. griseus*, of which three are reversible and show no essential difference from the parent strain; two may be considered as mutants which are not reversible to their parent strain (57).

8. Different strains of *S. griseus* vary greatly in their sensitivity to actinophages. Shimako (57) and others could not find, however, any strict specificity.

The original Waksman and Curtis culture of *S. griseus*, which was at first believed to produce no antibacterial substance, was recently (unpublished data by R. E. Robison, formerly of the Institute of Microbiology) found to form an antifungal substance of the candicidin type. It should be further noted that Kelner (31) irradiated this culture, kept since 1915 in the collection, and isolated from it a streptomycin-producing strain.

What conclusions can one draw from an analysis of the foregoing data on the variability of *S. griseus*? Should one become as pessimistic as Lieske (39) in his attitude toward the classification of actinomycetes as a whole? Or should one become as enthusiastic as Krassilnikov (35) in listing a large number of varieties? The latter point of view could lead to an absurd situation.

For example, one would have to consider the original Waksman and Curtis culture as *S. griseus* var. *candicidini*. Since the same culture yields, on irradiation, a streptomycin-producing strain, it would have to be designated as *S. griseus* var. *streptomycini*. The latter can give rise to two spontaneous variants, which would be designated as *S. griseus* var. *rhodomycetini* and *S. griseus* var. *sterilis*, and so on *ad infinitum*. Suppose the asporogenous culture on further cultivation on special media, such as soil, regained the property of producing the aerial mycelium? Would it then become another variety? Under these conditions, every streptomycin-producing company throughout the world would have its own variety of *S. griseus*, based on selection of highly potent streptomycin-producing strains; there would be as much justification to such a procedure as to the foregoing varieties.

It is much simpler to consider the group-species *S. griseus* as a population comprising different strains, each of which develops under special conditions of culture. Those variants that may become important either because of some special biochemical property or because of their practical utilization, should merely be listed, for the time being, by numbers or by special type of activity. At some future date, after much more additional basic information has been accumulated and the factors responsible for these variations are better understood, the whole problem may be re-examined.

It is interesting that, in examining the characteristics given by Krainsky to *A. griseus*, Baldacci (3, 4) was led to the conclusion that it belongs rather to *A. viridis*. As regards the description of *A. griseus* by Waksman and Curtis, Baldacci concludes that it appears advisable to take as definite the characteristics specified by the American authors and given in *Bergey's Manual*. Incidentally, the name "*griseus*" is scarcely an apt one for this species, as it is not "gray" but white to bottle-green in color when sporulating.

*Streptomyces scabies*. *S. scabies* represents another group-species that shows considerable variation. This has been emphasized by a number of investigators, particularly by Schaal (53), and need not be discussed in detail here. Beginning with the pioneering work of Thaxter (63) in 1890, and followed by numerous subsequent studies including those of Millard and Burr (44), two tendencies have been evident: (a) either to include all the strains in one species; or (b) to create a large number of new species, each of which is said to be capable of causing some form of potato scab. The difficulty of establishing pathogenicity by inoculating plants with various strains isolated from scabby potatoes leads one to question the validity of many of the species recorded, and to wonder whether all or at least some are not soil contaminants rather than causative agents of scab.

Undoubtedly, different strains or races of *S. scabies* are responsible for the infection of potatoes and mangels, as shown, for example, by the formation of different types of scab on different varieties of plants. No definite correlation has been found, however, between pathogenicity and cultural and other properties of the organism, although the variants were found to differ from the parent culture in pathogenicity. High nitrogen content of the medium appeared to inhibit production of aerial mycelium in the parasitic strains but not in the saprophytes. Of the twenty isolates tested by Schaal (53) on three different media, six did not produce any spirals but fourteen did. These spirals were of both sinistrorse and dextrorse types.

No attempt will be made here to examine in detail the many efforts to split this species into a number of subspecies, based either on morphological or cultural properties of the strains or upon the causation, or at least what was believed to be causation, of specific infections. Only further detailed genetic and cultural studies, combined with detailed infectivity studies, can resolve this complexity.

*Analysis of the history of Streptomyces flavus and allied species*. Widely distributed in nature are cultures of *Streptomyces* that produce a yellow to orange vegetative growth on either synthetic or organic media or both, and an aerial mycelium that ranges in color from white to gray. Such cultures have usually been designated by a variety of different names, in which the term "flavus" or one of its modifications has played a prominent part. This designation was used either as a specific name, of which there are several described by different investigators (32), or as a part of a more complex name, such as *alboflavus* and *griseoflavus*. A few of the cultures thus described as distinct species would undoubtedly be found on careful comparative study to belong to recognized species. Since some of the cultures belonging to this group have been found to

TABLE 2

*Identification of Streptomyces flaveolus by different investigators*

| | Waksman (64) | Takahashi (62) |
|---|---|---|
| Morphology | | |
| Sporophores | Numerous spirals on all media | Numerous spirals on synthetic media |
| Conidia | Oval to elliptical | Spherical or oval, 0.8 x 1.2$\mu$ |
| Czapek's agar | | |
| Vegetative growth | Light sulfur-yellow turning cadmium-yellow | Antimony-yellow to chamois colored |
| Aerial mycelium | White with ash gray patches | White, later smoke-gray |
| Soluble pigment | Empire-yellow | Buff-yellow |
| Calcium malate-$NH_4Cl$ agar | | |
| Vegetative growth | Cream-colored | Pale olive-buff to yellow ocher |
| Aerial mycelium | Mouse-gray, with white margin | Vinaceous-buff to light mouse-gray |
| Soluble pigment | None | None or faint yellowish |
| Nutrient agar | | |
| Vegetative growth | Wrinkled, white | Colorless to whitish, reverse cinnamon-buff |
| Aerial mycelium | Abundant, white | White |
| Soluble pigment | None | Golden yellow |
| Gelatin | | |
| Vegetative growth | Abundant yellowish pellicle | Wrinkled, yellow |
| Aerial mycelium | White | White |
| Soluble pigment | Golden to faint brown | Faint yellowish-brown |
| Liquefaction | Rapid | Medium |
| Potato plug | | |
| Vegetative growth | Wrinkled, cream-colored | Wrinkled, golden-yellow to orange |
| Aerial mycelium | White | White to seashell pink |
| Color of plug | Faint brown | Faint brownish |
| Glucose broth | | |
| Vegetative growth | Thin, yellow pellicle | Colonial-buff to honey-yellow |
| Aerial mycelium | White | White to smoke gray |
| Soluble pigment | Golden | Yellowish (golden yellow) |
| Milk | Rapid coagulation and peptonization | Rapid coagulation and strong peptonization |
| Nitrate reduction | Strong | Positive |
| Antibiotic production | Produces actinomycin | Produces flaveolin |

produce important antibiotics, there has been added recently a stimulus for the creation of new species. Unfortunately, minor variations in color intensity on certain media of unknown composition have often been considered as sufficient justification for this. On the other hand, there is no doubt that accurate identifications can be made and have been made on the basis of published descriptions alone. The following illustrations will suffice.

A culture of an organism was isolated by Takahashi (62) in Japan and identified by him as *S. flaveolus* Waksman. To indicate the justification of this identification, the details of the description of this culture are presented in table 2 side by side with those of the original type culture. These data, as reported in the published descriptions, show that in spite of minor variations in color characterization, in spite of quantitative differences in gelatin liquefaction and in nitrate reduction, and even in spite of differences in antibiotic production, the identification of the species appears to be correct.

The same is true for the characterization of *Streptomyces parvus*. The original culture of this organism, which was used as the basis for its description in *Bergey's Manual*, has died out in our collection. Recently, Dr. R. G. Benedict, of

TABLE 3

*Characterization of Streptomyces parvus*

| | Krainsky (33) | Bergey's 6th Edition (5) | New culture received from N.R.R.L.—3686 |
|---|---|---|---|
| Vegetative growth | | Golden yellow to brick red depending on composition of medium | Bright yellow |
| Aerial mycelium | White to gray to rose yellow depending on nitrogen source. Spores are more or less oval, 1.6μ in size | Poorly developed, rose-white. Sporophores produce spirals. Spores spherical to oval, 0.9 to 1.3 by 1.2 to 1.8μ | Long, straight hyphae; no spirals. Conidia short, oval |
| Synthetic agar | Colonies small, yellow in color, with light colored aerial mycelium* | Colonies small, yellow, with aerial mycelium light yellow | Thin, yellow growth. Thin white to yellow aerial mycelium. Bright yellow soluble pigment |
| Nutrient agar | Yellow growth. Aerial mycelium appears late | | Yellow growth; abundant white with grayish tinged aerial mycelium. Bright yellow soluble pigment |
| Glucose-asparagine agar | | | Yellow growth. White to gray aerial mycelium. Golden soluble pigment |
| Glucose agar | Aerial mycelium light yellow; appears late | Colonies small, yellow, with aerial mycelium light yellow | |
| Gelatin | Colonies flat or concave, yellow in color. Gelatin slowly liquefied | Colonies yellow. Liquefaction medium | Cream-colored growth dropping to bottom. Good liquefaction. Bright-yellow soluble pigment |
| Potato | Colonies yellow, aerial mycelium white | | Abundant wrinkled brownish yellow growth. Abundant sulfur-yellow aerial mycelium. No soluble pigment |
| Cellulose | White surface growth | Growth good | |
| Remarks | Produces diastase; reduces nitrate slowly; strong proteolytic activity | Produces actinomycin; reduces nitrate slightly | |

* Calcium malate agar.

the Northern Utilization Research Laboratory of the USDA, isolated a fresh culture from a sample of soil collected in the Ayensu basin of the Gold Coast of West Africa, which he identified as *S. parvus*. A comparison was made in our laboratory between the culture originally isolated and described by Krainsky, the culture isolated by Waksman and Curtis and reported in *Bergey's Manual*, and the new culture of Benedict. The results are presented in table 3. They point definitely to the identity of the three cultures, thus proving again that accurate identification can be made by comparing a freshly isolated culture with written descriptions of type isolates.

Finally a comparison may now be presented of two published descriptions of *S. flavus* and of *S. griseoflavus* together with recent descriptions of two freshly isolated cultures that have been raised to the status of new species, namely, *S. aureo-*

TABLE 4

*Characteristics of Streptomyces flavus and allied strains*

| | *S. flavus* (Bergey) (5) | *S. griseoflavus* (Bergey) (5) | *S. aureofaciens* (18) | *S. rimosus* (60) |
|---|---|---|---|---|
| Morphology | | | | |
| Sporophores | Straight, much branched, no spirals | Straight, no spirals | Straight, flexuous. No spirals as a rule; occasional loose spirals | Numerous spirals |
| Conidia | Oval | | Spherical to oval, 1.5μ long | Short, cylindrical 0.6 to 0.7 x 0.8 to 1.4μ |
| Czapek's agar | | | | |
| Vegetative growth | Yellow to sulfur yellow | Reddish brown to orange | Heavy cream-colored, becoming yellowish brown† | Submerged, colorless‡ |
| Aerial mycelium | Straw yellow | White | Abundant, at first white, turning mouse gray to brownish-gray from center to periphery | None |
| Soluble pigment | | Faint yellowish | Brownish | None |
| Calcium malate-$NH_4Cl$ agar | | | | |
| Vegetative growth | Thin, cream-colored turning orange-brownish* | | Hyaline, changing to orange yellow or reddish brown§ | Poor, flat, yellowish |
| Aerial mycelium | White, scanty | | Abundant, white, changing to deep gray or dark gray; tawny reverse | None |
| Soluble pigment | Yellowish golden | | Faint yellowish or absent | None |
| Nutrient agar | | | | |
| Vegetative growth | Cream-colored, lichenoid | Cream colored | Good, light brownish | Cream-colored to brownish |
| Aerial mycelium | White to light-gray | White | None | None or white to gray white† |
| Soluble pigment | None | None | None | Faint yellowish or none† |
| Gelatin | | | | |
| Vegetative growth | Yellowish | Cream-colored to brownish | | Moderate growth |
| Aerial mycelium | None | White | | White |
| Liquefaction | Positive | Slow | None | Medium to good |
| Soluble pigment | Faint yellowish | Faint yellowish | | None to yellowish† |

* Glucose-asparagine agar.

† Observations made in our laboratory (not in original description).

‡ It should be noted here that an earlier description of this culture on Czapek's agar (19) was incorrectly labelled; this medium was made up at this time with glucose in place of sucrose, which explains the difference between the previous and present observations.

§ Asparagine-meat extract agar.

TABLE 4—*Continued*

| | *S. flavus* (Bergey) (5) | *S. griseoflavus* (Bergey) (5) | *S. aureofaciens* (18) | *S. rimosus* (60) |
|---|---|---|---|---|
| Potato plug | | | | |
| Vegetative growth | Abundant, lichenoid, brownish to greenish olive | Lichenoid, brownish to reddish-brown | Wrinkled, orange yellow | Wrinkled, ochroid growth |
| Aerial mycelium | White to gray | White to gray | | Whitish to drab |
| Color of plug | Brownish or none | None | Unchanged | Yellowish brown pigment or none† |
| Yeast-glucose agar | | | | |
| Vegetative growth | Rapid, lichenoid, brownish | Cream-colored to brownish | Heavy, cream-colored*† | Good, yellowish* |
| Aerial mycelium | White, later grayish | White to grayish | Abundant white to deep gray or dark gray | Pallid drab |
| Soluble pigment | Yellow | Yellowish | None | Yellowish |
| Milk | | | | |
| Vegetative growth | Yellowish | Cream-colored to yellowish | Yellow brown, limited | Abundant grayish growth |
| Aerial mycelium | White | Thin, white | | White |
| Coagulation and peptonization | Coagulation and peptonization | No coagulation, rapid peptonization | None | No peptonization |
| Nitrate reduction | Slight | Positive | | Weak to strong |
| Production of enzymes and antibiotics | Produces an antibacterial agent | | Produces chlortetracycline | Produces oxytetracycline and rimocidin |

*faciens* and *S. rimosus*. The last two are important producers of antibiotics. Such a comparison will make it possible to determine what justification there was in establishing these two new species.

The results presented in table 4 show that *S. aureofaciens* and *S. rimosus* are sufficiently different from *S. flavus* and *S. griseoflavus* to warrant the creation of new species. *S. aureofaciens* is characterized by a deep gray aerial mycelium, by a lack of or limited spiral formation, by poor proteolysis, as shown by limited action upon gelatin and milk, and by poor growth on nutrient agar. These properties differentiate it sharply from those of the two older cultures. *S. rimosus* is characterized by poor growth on Czapek's agar and by the formation of abundant spirals in its aerial mycelium. These properties, together with certain other morphological and cultural differences between this culture and the two older cultures, justify its creation as a separate species, especially because of its ability to produce an important new antibiotic.

However, in view of the great variability of these organisms and in view of the temptation to establish separate species on the basis of minor differences in pigmentation, any attempt to create such new species must be considered critically. Attention may be called, for example, to *Streptomyces armillatus* (43). On the basis of the description only, this culture appears to be sufficiently close to *S. rimosus* to throw doubt upon its distinct identity. Like the latter, it produces spirals in its aerial mycelium; it forms very poor growth on synthetic agar, without any aerial mycelium and without pigmentation; on nutrient agar, it produces yellow-gray growth with poorly developed white aerial mycelium and no soluble pigment; on potato plug, it produces yellow-gray growth with a faint brownish soluble pigment; on gelatin it forms a surface growth

with white aerial mycelium, with a yellowish or brownish soluble pigment and good liquefaction of the gelatin; on milk it produces good grayish growth. These characteristics, together with the ability of the culture to produce oxytetracycline, would definitely place it in the *S. rimosus* species. On the other hand, emphasis is laid upon the fact that it produces a flat colony, hardly folded and not cracking like those of *S. rimosus*; it forms concentric circles in the aerial mycelium, a variable property, indeed. It does not form nitrite from nitrates, it does not hydrolyze starch; both of these properties are hardly sufficient to justify the recognition of *S. armillatus* as a new species, especially since one wonders how constant these characters were found to be.

## SUMMARY AND CONCLUSIONS

At present various morphological, cultural, and biochemical properties are known which make it possible to establish definitely certain distinct species among the actinomycetes. Some of these characters are constant within certain conditions of nutrition and environment, others are variable. Certain additional properties may be required in order to establish the degree of variation of a culture before it can be recognized as a new species.

Certain categories of relationships among the actinomycetes must be taken into consideration in order to establish definitely the systematic position of a given culture. These may be briefly summarized as follows:

1. On the basis of all the accumulated evidence, actinomycetes are shown to belong definitely to the bacteria.

2. The position of the true actinomycetes to the closely related bacterial forms, notably the mycobacteria and corynebacteria, must be recognized; this is true especially of certain nocardial types.

3. The generic interrelationships among the actinomycetes are highly significant. The separation of members of the genus *Streptomyces* from those of *Nocardia* is difficult, especially when one is dealing with non-sporulating forms of the first and sporulating forms of the second. The recent addition of two new genera, *Actinoplanes* and *Streptosporangium*, and the recognition of *Thermoactinomyces* as a separate genus, add further problems to these generic interrelationships. Very little can still be said of other proposed generic names, such as *Chainia* and *Jensenia*.

4. Within each genus certain groups, species group, or series must be recognized. A combination of morphological and cultural properties permits the establishment of species-groups. Some of them comprise a large number of forms with many variable characteristics. This is true, for example, within the genus *Streptomyces*, of such groups as *albus*, *aureus*, *griseus*, *lavendulae*, *flavus*, *coelicolor*, *fradiae*, and *scabies*.

5. Differentiation of individual species within each group is based upon a combination of cultural and biochemical properties. The production of specific antibiotics and utilization of different sugars are ample illustrations of this.

6. Cognizance of the strains and varieties within each species must finally be taken into account. This may be based upon certain qualitative properties, such as sensitivity to phages, or quantitative properties, such as production of a given antibiotic, vitamin or enzyme, or sensitivity to a given antibiotic.

A detailed examination has been made of the characteristic properties of several well defined species of *Streptomyces* as described in the literature. On comparing these descriptions with the properties of freshly isolated cultures, one may be justified in drawing certain general conclusions pretaining to speciation of actinomycetes:

1. A group of morphological and cultural properties, obtained by growing the cultures on several well defined synthetic and organic media, are now available which are sufficient for fairly close identification of the majority of newly isolated cultures with previously described species.

2. Minor quantitative or even certain qualitative variations in the properties of such cultures, when compared with descriptions of recognized species, are not sufficient in many cases to justify the creation of new species.

3. Cultures showing specific biochemical reactions, such as production of new antibiotics, may be raised to the status of "species" only when accompanied by other differences, notably morphological or cultural, or both. Otherwise, they must be considered merely as varieties.

4. The selection of certain specific media, the establishment of standard conditions of culture, the use of type species, especially those that have

not degenerated on continuous cultivation under artificial conditions, will contribute further to the uniformity of speciation among the actinomycetes.

5. Consideration must always be given to variations, both those that are natural and those that are produced during degeneration on artificial media. One is not justified in assigning a new species name until a detailed study has been made of the limits of variation of the culture, and until it has become recognized, on the basis of morphological, cultural, and biochemical properties to be sufficiently different from well-described species to deserve a new name.

## APPENDIX I

### *Color Designations for Describing Actinomycetes According to Lindenbein (40)*

1. *White:* a. snow white (*niveus*), b. glossy white (*candidus*), c. silver white (*argenteus*), d. milk white (*lacteus*), e. chalk white (*cretaceus*), f. gray white (*farinaceus*); also cream, egg-shell, and ivory.

2. *Violet:* a. bluish violet (*violaceus*), b. reddish violet (*lilaceus*), also lavender, mauve, purple.

3. *Blue:* a. dark blue (*caeruleus*), b. cornflower blue (*cyaneus*), c. sky blue (*azureus*), d. gray blue (*caesius*), e. yellowish blue (*lividus*).

4. *Green:* a. grass green (*viridis*), b. emerald green (*smaragdinus*), c. blue green (*glaucus*), d. forest green (*prasinus*), e. olive green (*olivaceus*).

5. *Yellow:* a. light yellow (*flavus*), b. deep yellow (*luteus*), c. citron yellow (*citrinus*), d. golden yellow (*aureus*), e. sulfur yellow (*sulfureus*), f. white yellow (*stramineus*), g. brownish yellow (*gilvus*), h. egg-yolk yellow (*vitellinus*), i. pale yellow (*luridus*), k. greenish yellow (*galbus*).

6. *Orange:* a. light orange (*aurantiacus*), b. dark reddish-orange (*croceus*).

7. *Red:* a. dark red (*ruber*), b. carmine red (*puniceus*), c. scarlet red (*coccineus*), d. fire red (*igneus*), e. pale carmine (*roseus*), f. flesh red (*carneus, incarnatus*), g. purple red (*purpureus*), h. cinnabar red (*cinnabarinus*), i. lead red (*miniatus*), k. brick red (*lateritius*), l. blood red (*sanguineus*), m. brownish copper red (*cupreus*), n. light yellow red (*rutilus*); also pink, coral pink, rose, and wine-colored (*vinaceus*).

8. *Brown:* a. light brown (*brunneus*), b. dark brown (*umbrinus*), c. chestnut brown (*badius*), d. reddish brown (*fuscus*), e. yellow rusty brown (*ferrugineus*), f. greenish brown (*hepaticus*), g. cinnamon brown (*cinnamomeus*); also beige, tan, and ocher.

9. *Gray:* a. greenish gray (*griseus*), b. ash gray (*cinereus*), c. white gray (*incanus*), d. brownish gray (*fumigatus*), e. reddish gray (*murinus*), f. bluish gray (*plumbeus*).

10. *Black:* a. gray black (*niger*), b. coal black (*ater*), c. brownish pitch black (*piceus*), d. greenish-jet-black (*coracinus*), e. blue black (*atramentarius*).

## APPENDIX II

### *Most Important Media Used for the Study of Actinomycetes*

1. *Czapek's agar:*

| | |
|---|---|
| Sucrose | 30.0 g |
| $NaNO_3$ | 2.0 g |
| $K_2HPO_4$ | 1.0 g |
| $MgSO_4 \cdot 7H_2O$ | 0.5 g |
| KCl | 0.5 g |
| $FeSO_4$ | 0.01 g |
| Agar | 15.0 g |
| Distilled water | 1.0 l |

pH 7.0 to 7.3

Glycerol or glucose is often substituted for sucrose, giving glycerol-, or glucose-Czapek's agar.

2. *Glucose-asparagine agar:*

| | |
|---|---|
| Glucose | 10.0 g |
| Asparagine | 0.5 g |
| $K_2HPO_4$ | 0.5 g |
| Agar | 15.0 g |
| Distilled water | 1.0 l |

pH 6.8

In some cases, 2 g meat-extract is added per liter of medium, and tap water is used.

3. *Glycerol-asparagine agar:*

| | |
|---|---|
| Glycerol | 35.0 ml |
| NaCl | 5.0 g |
| $CaCl_2$ | 0.1 g |
| $MgSO_4$ | 0.3 g |
| $K_2HPO_4$ | 2.5 g |
| Ammonium lactate | 6.5 g |
| Sodium asparaginate | 3.5 g |
| Agar | 20.0 g |
| Distilled water | 1.0 l |

This medium is often spoken of as Ushinsky's, especially when used as a solution, without agar.

4. *Ca-malate agar:*

| | |
|---|---|
| Ca-malate | 10.0 g |
| $K_2HPO_4$ | 0.5 g |
| $NH_4Cl$ | 0.5 g |
| Agar | 15.0 g |
| Distilled water | 1.0 l |

Glycerol, 10 g, is often added to this medium.

5. *Tyrosine agar:*

| | |
|---|---|
| Glucose | 10.0 g |
| Tyrosine | 1.0 g |
| $(NH_4)_2SO_4$ | 0.5 g |
| $K_2HPO_4$ | 0.5 g |

| | |
|---|---|
| Agar | 15.0 g |
| Distilled water | 1.0 l |

Reaction made neutral with NaOH.

6. *Nutrient or meat-peptone agar:*

| | |
|---|---|
| Peptone | 5.0 g |
| Meat extract | 5.0 g |
| NaCl | 5.0 g |
| Agar | 15 to 20 g |
| Distilled water | 1.0 l |

pH 7.2 to 7.4

Frequently 10 g peptone is used. 10.0 g glucose or 15.0 g of glycerol may be added to give glucose- or glycerol-peptone agar. In a liquid condition without agar, these media are nutrient broth, glucose-peptone broth, or glycerol-peptone broth, respectively.

7. *Nutrient salt agar:*

| | |
|---|---|
| Peptone | 5.0 g |
| Meat extract | 5.0 g |
| Glucose | 10.0 g |
| $K_2HPO_4$ | 1.0 g |
| $MgSO_4 \cdot 7H_2O$ | 0.5 g |
| KCl | 0.5 g |
| Distilled water | 1.0 l |

The organic nutrients may be reduced to a half or even a tenth of above concentration, in order to favor production of aerial mycelium in some cultures of actinomycetes.

8. *Plain gelatin:*

| | |
|---|---|
| Gelatin | 100.0 to 200.0 g |
| Tap water | 1.0 l |

Adjust to pH 7.4

Sterilize 30 minutes at 110 C.

9. *Peptone gelatin:*

| | |
|---|---|
| Peptone | 5.0 g |
| Glucose | 20.0 g |
| Gelatin | 100.0 to 200.0 g |
| Tap water | 1.0 l |

Adjust to pH 7.2

Sterilize 30 minutes at 110 C.

10. *Starch agar:*

| | |
|---|---|
| Soluble starch | 10.0 g |
| $NaNO_3$ | 1.0 g |
| $K_2HPO_4$ | 0.3 g |
| NaCl | 0.5 g |
| $MgCO_3$ | 1.0 g |
| Agar | 15.0 g |
| Distilled water | 1.0 l |

3 g $CaCO_3$ and 1 g $MgSO_4 \cdot 7H_2O$ may replace the $MgCO_3$. 2 g $(NH_2)_2SO_4$ and 0.05 g asparagine may be used to replace the nitrate.

11. *Egg-albumen agar:*

| | |
|---|---|
| Glucose | 10.0 g |
| Egg albumen | 0.15 g |
| $K_2HPO_4$ | 0.5 g |
| $MgSO_4 \cdot 7H_2O$ | 0.2 g |
| $Fe_2(SO_4)_3$ | Trace |
| Agar | 15.0 g |
| Distilled water | 1.0 l |

Egg albumen is first dissolved in water and made neutral to phenolphthalein with N/10 NaOH.

12. *Potato-glucose agar:*

| | |
|---|---|
| Peeled potatoes | 200.0 or 300.0 g |
| Glucose | 5.0 g |
| Agar | 20.0 g |
| Tap or distilled water | 1.0 l |

pH 6.8 to 7.2

13. *Yeast-peptone agar:*

| | |
|---|---|
| Meat extract | 4.0 g |
| Peptone | 4.0 g |
| Yeast extract | 1.0 g |
| Glucose | 10.0 g |
| NaCl | 2.5 g |
| Distilled water | 1.0 l |

This medium is often known as Emerson's. Various modifications of this medium are used. The concentration of the first two constituents may be reduced to 1.0 g per liter; the NaCl may be left out; the peptone may be replaced by enzymatic casein hydrolyzate (N-Z-Amine A), thus giving Bennett's medium.

14. *Yeast-glucose agar:*

| | |
|---|---|
| Yeast extract | 10.0 g |
| Glucose | 10.0 g |
| Agar | 15.0 g |
| Tap water | 1.0 l |

pH 6.8

Malt extract, 10.0 g, is sometimes added to this medium.

15. *Oatmeal agar:*

| | |
|---|---|
| Rolled oats | 65.0 g |
| Distilled water | 1.0 l |

Cook to thin gruel in double boiler, filter through several layers of cheesecloth, and make up to a liter while still hot. Add 2 per cent agar and sterilize.

16. *Soil extract agar:*

| | |
|---|---|
| Beef extract | 3.0 g |
| Peptone | 5.0 g |
| Agar | 15.0 g |
| Soil extract | 1.0 l |

pH 7.0

The soil extract is prepared by treating 1 kg garden soil with 2.5 liters of tap water for one hour in autoclave at 15 lb pressure. Filter hot. Add talc for clarification, if necessary.

17. *Carbon nutrition medium:*

| | |
|---|---|
| $(NH_4)_2SO_4$ | 2.64 g |
| $KH_2PO_4$ | 2.38 g |
| $K_2HPO_4$ | 5.65 g |
| $MgSO_4 \cdot 7H_2O$ | 1.00 g |
| $CuSO_4 \cdot 5H_2O$ | 0.0064 g |
| $FeSO_4 \cdot 7H_2O$ | 0.0011 g |
| $MnCl_2 \cdot 4H_2O$ | 0.0079 g |

| | | |
|---|---|---|
| $ZnSO_4 \cdot 7H_2O$ | 0.0015 | g |
| Carbon source | 10.00 | g |
| Agar | 15.00 | g |
| Distilled water | 1.0 | l |

Some carbon compounds may have to be sterilized separately either by filtration or by heating in aqueous solution. This medium used by Pridham and Gottlieb (50) has found general application in the study of utilization by actinomycetes of different carbon sources.

18. *Cellulose medium:*
Filter paper saturated with Czapek's solution, free from sucrose.
19. *Potato plug:*
Distilled water added.
20. *Carrot plug:*
Distilled water added.
21. *Litmus milk:*
Skim milk powder is used. Brom-cresol milk may also be used.

## APPENDIX III

### *Classification of Species Belonging to the Genus Streptomyces*

*1. Waksman and Henrici System.*

This system is based primarily upon the ecology of the organisms, production of soluble pigments in organic and synthetic media, and manner of sporulation.

I. Saprophytes; psychrophilic to mesophilic.
  1. Soluble pigment on organic media other than brown or faint brown.
    a. Pigment absent or faint brown only.
    b. Pigment blue.
    c. Pigment at first green, becoming brown, etc.
  2. Soluble brown pigment on organic media.
  3. No soluble pigment produced in organic media.
    a. Proteolytic action strong.
    b. Proteolytic action limited.
    c. Proteolytic action very weak.

II. Saprophytes; thermophilic.
  1. Yellowish growth on potato; diastatic.
  2. Dark-colored growth on potato abundant, nondiastatic.
  3. Thermotolerant cultures.

III. Plant parasites or cultures isolated from diseased plants or from soil in which diseased plants were grown.
  1. Isolated from potato scab or from soil in which scabby potatoes were grown.
  2. Grow on or isolated from sweet potatoes.
  3. Attack or isolated from scab on mangels and sugar beets.

IV. Isolated from animal tissues; in the animal body, hyphae often show clavate enlargements at the ends.
  1. Limited proteolytic action.
  2. Strong proteolytic action.

V. Produce only vegetative growth and no aerial mycelium.

*2. Krassilnikov system.*

This is based upon manner of sporulation, shape of spores, pigmentation of cultures.

I. Sporophores branching monopodially.
  1. Spiral-shaped sporophores, produced on hyphae of aerial mycelium.
    a. Spores spherical or oval.
      $a^1$. Cultures colorless, not producing any pigmentation.
      $b^1$. Cultures pigmented blue, etc.
    b. Spores cylindrical or elongated.
      $a^1$. Cultures colorless.
      $b^1$. Cultures red, sporophores mostly straight, etc.
  2. Sporophores straight or wavy, but not spiral.
    a. Spores produced by means of fragmentation of plasma within cells.
      $a^1$. Spores spherical or oval.
      $b^1$. Spores cylindrical or elongated.

II. Sporophores produced in whorls.
  1. Sporophores straight.
  2. Sporophores spiral-shaped.
    a. Spores spherical, oval.
    b. Spores cylindrical, elongated.

*3. Combination of the two systems.*

Hesseltine, Benedict, and Pridham (25) combined the foregoing systems, emphasizing the morphological types as the major basis for the separation of the genera. The five basic types were subdivided into a number of subtypes on the basis of cultural properties, pigmentation of spores, and other criteria.

1. Conidiophores not restricted in length, bearing fertile branches in verticils (whorls), with conidia more or less strongly attached.
  a. Fertile branches in simple verticils, branches not ending in spirals.
  b. Fertile branches in simple verticils, branches ending in spirals.
  c. Fertile branches with compound verticils, branches not ending in spirals.
2. Conidiophores with branches all straight, never ending in spirals; verticils absent.
3. Conidiophores predominantly in tufts, never verticillate; outline of branches flexuous and irregular.
4. Conidiophores with branches ending in spirals; verticils absent; conidiophores either as long stalks bearing very short branches, or as short stalks bearing branches irregularly.

a. Branches ending in open spirals with many turns.
b. Branches ending in closed spirals with few turns, thus appearing as tight knots.

5. Conidiophores with long and straight branches with spirals of large diameter at their ends; spirals usually with only a few turns; never verticillate.

No strains have been observed in which the conidiophores are unbranched, except where they are growing under unfavorable conditions or in degenerated type cultures.

*4. Recognition of group-species.*

Before a detailed classification of the species based upon these systems is given, emphasis may be laid upon the group-concept, especially as pertaining to the genus *Streptomyces*. Waksman recognized the significance of the group-species in his early studies (68). More recently Baldacci *et al.* (4) further emphasized the need for group or "series" recognition, using the following basis:

| *Genus* | *Species* |
|---|---|
| Presence or absence of spores | Enzymatic activity |
| Arrangement of the spores (single or in series)[2] | Antibiotic activity |
| Ramification and breaking up of the vegetative mycelium | Soluble pigments spreading through the substratum, in relation to nutrition and pH |

Key to the species of *Streptomyces* on the basis of series (Baldacci):

A. Colorless[3] vegetative mycelium.
1. Aerial mycelium—white. Series *albus*
2. Aerial mycelium—sea green. Series *griseus*
3. Aerial mycelium—green-azure. Series *viridis*
4. Aerial mycelium—azure. Series *caeruleus*
5. Aerial mycelium—white/wine/lavender. Series *lavendulae*
6. Aerial mycelium—light pink. Series *roseus*
7. Aerial mycelium—gray. Series *diastaticus*

B. Colored vegetative mycelium
I. Yellow vegetative mycelium
8. Vegetative mycelium—lemon yellow to custard yellow.
Aerial mycelium—greyish white. Series *aureus*
9. Vegetative mycelium—reddish yellow-green.
Aerial mycelium—red/white. Series *madurae*
10. Vegetative mycelium—orange yellow.
Aerial mycelium—reddish gray. Series *fradiae*
11. Vegetative mycelium—golden yellow.
Aerial mycelium—white. Series *albido-flavus*
II. Vegetative mycelium—brown to black
12. Aerial mycelium—gray. Series *antibioticus*
13. Aerial mycelium—grayish white. Series *scabies*
14. Aerial mycelium—yellow. Series *sulphureus*
15. Aerial mycelium—greenish gray. Series *intermedius*
III. Vegetative mycelium—maroon
16. Aerial mycelium—gray. Series *rimosus*
IV. Vegetative mycelium—pink
17. Aerial mycelium—white. Series *bostroemi*
V. Vegetative mycelium—red
18. Aerial mycelium—white. Series *albosporeus*
19. Aerial mycelium—pink red. Series *rubriraeticuli*
20. Aerial mycelium—black. Series *melanosporeus*
VI. Vegetative mycelium—bluish violet-red
21. Aerial mycelium—white with various tints. Series *violaceus*

[2] Characteristics of the colonies under culture, and color of the vegetative mycelium and aerial mycelium.

[3] The term "colorless" refers to that mycelium which takes on the color shades of the substratum from which it is developing.

## REFERENCES

1. AOKI, M. 1935–1936 Agglutinatorische Untersuchung von Aktinomyzeten. Z. Immunitätsforsch., **86**, 518–524; **87**, 196–199, 200–201; **88**, 60–62.
2. AVERY, R. J., AND BLANK, F. 1954 On the chemical composition of the actinomycetales and its relation to their systematic position. Can. J. Microbiol., **1**, 140–143.
3. BALDACCI, E. 1947 Die Systematik der Actinomyceten. Mycopathologia, **4**, 60–84.
4. BALDACCI, E., SPALLA, C., AND GREIN, A. 1955. The classification of the *Actinomyces* species (= *Streptomyces*). Arch. Mikrobiol., **20**, 347–357, 1954, Giorn. Microbiol., **1**, 28–34.

5. *Bergey's manual of determinative bacteriology.* 1st ed. 1923; 5th ed. 1939; 6th ed. 1948; 7th ed. (To be published). The Williams & Wilkins Co., Baltimore, Md.
6. BUCHANAN, R. E. 1954 Some interrelationships of speciation, type preservation, and nomenclature in bacteria. Ann. N. Y. Acad. Sci., **60**, 6–15.
7. BURKHOLDER, P. R., SUN, S. H., EHRLICH, J., AND ANDERSON, J. L. 1954 Criteria of speciation in the genus *Streptomyces*. Ann. N. Y. Acad. Sci., **60**, 102–123.
8a. CARVAJAL, F. 1946 Studies on the structure of *Streptomyces griseus*. Mycologia, **38**, 387–395.
8b. CARVAJAL, F. 1946 Biologic strains of *Streptomyces griseus*. Mycologia, **38**, 596–607.
9. CARVAJAL, F. 1953 Studies on mycophagy with a polyvalent phage from *Streptomyces griseus*. Bacteriol. Proc., p 40.
10. COHN, F. 1875 Untersuchungen über Bakterien. II. Beitr. Biol. Pflanz., **1** (3), 186–188.
11. COUCH, J. N. 1950 *Actinoplanes*, a new genus of *Actinomycetales*. J. Elisha Mitchell Sci. Soc., **66**, 87–92.
12. COUCH, J. N. 1954 The genus *Actinoplanes* and its relatives. Trans. N. Y. Acad. Sci., **16**, 315–318.
13. COUCH, J. N. 1955 A new genus and family of the *Actinomycetales*, with a revision of the genus *Actinoplanes*. J. Elisha Mitchell Sci. Soc., **71**, 148–155, 269.
14. COWAN, S. T. 1955 The principles of microbial classification. Introduction: The philosophy of classification. J. Gen. Microbiol., **12**, 314–321.
15. DOMEC, T. 1892. Contribution à l'étude de la morphologie de l'*Actinomyces*. Arch. med. exptl. anat. pathol., **4**, 104–113.
16. DRECHSLER, C. 1919 Morphology of genus *Actinomyces*. Botan. Gaz., **67**, 65–83, 147–164.
17. DULANEY, E. L. 1954 Induced mutation and strain selection in some industrially important microorganisms. Ann. N. Y. Acad. Sci., **60**, 155–167.
18. DUGGAR, B. M. 1948 Aureomycin: a product of the continuing search for new antibiotics. Ann. N. Y. Acad. Sci., **51**, 177–181; U. S. Patent 2,482,055, September 13, 1949.
19. DUGGAR, B. M., BACKUS, E. J., AND CAMPBELL, T. H. 1954 Types of variation in actinomycetes. Ann. N. Y. Acad. Sci., **60**, 71–85.
20. GORDON, R. E., AND SMITH, M. M. 1955 Proposed group of characters for the separation of *Streptomyces* and *Nocardia*. J. Bacteriol., **69**, 147–150.
21. GUILLIERMOND, A., AND MANGENOT, G. 1946 *Précis de biologie végétale*. 2nd ed. Masson et Cie, Paris.
22. HAASS, E. 1906 Beitrag zur Kenntnis der Aktinomyceten. Centr. Bakteriol. Parasitenk. I. Abt. Orig., **40**, 180–186.
23. HAGEDORN, H. 1955 Beitrag zur Cytologie und Morphologie der Actinomyceten. Zentr. Bakteriol. Parasitenk. II Abt., **107**, 353–375.
24. HARZ, C. O. 1877–1878 *Actinomyces bovis*, ein neuer Schimmel in den Geweben des Rindes. Jaherb. Tierarzneisch. München. p 125–140.
25. HESSELTINE, C. W., BENEDICT, R. G., AND PRIDHAM, T. G. 1954 Useful criteria for species differentiation in the genus *Streptomyces*. Ann. N. Y. Acad. Sci., **60**, 136–151.
26. HITCHCOCK, A. S. 1925 Methods of descriptive systematic botany. John Wiley & Sons, Inc., New York.
27. HORVATH, J. 1954 Contributions to the biology of quantitative changes in antibiotic production, based upon investigations on a *Streptomyces* species. Acta Microbiol., **1**, 131–140.
28. HORVÁTH, J., MARTON, M., AND OROSZLÁN, I. 1954 Vegetative hydridisationsversuche an Streptomyces. Acta Microbiol., **2**, 21–37.
29. HUCKER, G. J., AND PEDERSON, C. S. 1931–32 A study of the physiology and classification of the genus *Leuconostoc*. Zentr. Bakteriol. Parasitenk. II Abt., **85**, 65–114.
30. JONES, K. L. 1949, 1954 Fresh isolates of actinomycetes in which the presence of sporogenous aerial mycelia is a fluctuating characteristic. J. Bacteriol., **57**, 141–145; Ann. N. Y. Acad. Sci., **60**, 124–135.
31. KELNER, A. 1949 Studies on the genetics of antibiotic formation: The induction of antibiotic-forming mutants in actinomycetes. J. Bacteriol., **57**, 73–92.
32. KOCHI, M., RUIGH, W. L., ACKER, R. F., LECHEVALIER, H. A., AND WAKSMAN, S. A. 1952 Antibiotic producing properties of *Streptomyces* 3560, a member of the *S. flavus* group. Proc. Natl. Acad. Sci. U. S., **38**, 383–391.
33. KRAINSKY, A. 1914 Die Aktinomyceten und ihre Bedeutung in der Natur. Zentr. Bakteriol. Parasitenk. II Abt., **41**, 649–688.

34. KRASSILNIKOV, N. A. 1941 The guide to the ray fungi. Actinomycetales. Acad. Nauk. USSR, Moskau (Translated title).
35. KRASSILNIKOV, N. A. 1949 Determination of bacteria and actinomycetes. Acad. Sci. USSR Institut of Microbiology, Moskau (Translated title).
36. KRASSILNIKOV, N. A. 1950 Actinomycete-antagonists and antibiotic substances. Acad. Nauk. USSR, Moskau (Translated title).
37. KÜSTER, E. 1953, 1955 Beitrag zur Genese und Morphologie der Streptomyceten-sporen. Atti del VI Congresso Intern. Microbiol. Roma, **1**, 114–116, Zentr. Bakteriol. Parasitenk. II Abt., **108**, 376–382.
38. LANGERON, M. 1952 *Précis de mycologie.* 2nd ed. Masson et Cie, Paris.
39. LIESKE, R. 1921 *Morphologie und Biologie der Strahlenpilze.* Gebruder Bornstrager, Leipzig.
40. LINDENBEIN, W. 1952 Über einige chemish interessante Aktinomycetenstämme und ihre Klassifizierung. Arch. Mikrobiol., **17**, 361–383.
41. LÖHNIS, F. 1921 *Studies upon the life cycles of the bacteria.* Memoirs of the National Academy of Sciences. Vol. XVI, 2nd Memoir, Part I.
42. LUDWIG, E. H., AND HUTCHINSON, W. G. 1949 A serological study of selected species of actinomycetes. J. Bacteriol., **58**, 89–101.
43. MANCY-COURTILLET, D., AND PINNERT-SINDICO, S. 1954 Une nouvelle espèce de Streptomyces: *Streptomyces armillatus.* Ann. inst. Pasteur, **87**, 580–584.
44. MILLARD, W. A., AND BURR, S. 1926 A study of twenty-four strains of *Actinomyces* and their relation to types of common scab of potato. Ann. Appl. Biol., **13**, 580–644.
45. NÄGELI, C. V. 1877 *Die niederen Pilze.* R. Oldenbourg, Munich, Germany.
46. NOVAL, J. J., AND NICKERSON, W. J. 1956 Exocellular keratinase from a *Streptomyces* sp. Bacteriol. Proc., p 125.
47. OCHOA, A. G., AND HOYOS, A. V. 1953 Relaciones serologicas de los principales actinomycetes patogenos. Rev. inst. salubridad y enfermedad trop. Mex., **13**, 177–187.
48. ØRSKOV, J. 1923 *Investigations into the morphology of the ray fungi.* Levin and Munksgaard, Copenhagen, Denmark.
49. PANTIN, C. F. A. 1954 The recognition of species. Science Progr., **42**, 587–598.
50. PRIDHAM, T. G., AND GOTTLIEB, D. 1948 The utilization of carbon compounds by some *Actinomycetales* as an aid for species determination. J. Bacteriol., **56**, 107–114.
51. RAPER, K. B. 1954 Introduction to symposium "Speciation and variation in asexual fungi." Ann. N. Y. Acad. Sci., **60**, 3–5.
52. ROMANO, A. H., AND SOHLER, A. 1956 A comparison of the cell wall composition of *Streptomyces* and *Nocardia.* J. Bacteriol., **72**, 865–868.
53. SCHAAL, L. A. 1944 Variation and physiologic specialization in common scab fungus (*Actinomyces scabies*). Agr. Research, **69**, 169–186.
54. SCHATZ, A., BUGIE, E., AND WAKSMAN, S. A. 1944 Streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative bacteria. Proc. Soc. Exptl. Biol. Med., **55**, 66–69.
55. SCHATZ, A., AND WAKSMAN, S. A. 1945 Strain specificity and production of antibiotic substances. IV. Variations among actinomycetes, with special reference to *Actinomyces griseus.* Proc. Natl. Acad. Sci. U. S., **31**, 129–137.
56. SERMONTI, G., AND SPADA-SERMONTI, I. 1955 Genetic recombination in *Streptomyces.* Nature, **176**, 121.
57. SHIMAKO, O. 1951 Studies on the variability of *Streptomyces.* I. Natural variation of *Streptomyces griseus.* J. Antibiotics (Japan), *Suppl. A*, **4**, 1–6.
58. SLACK, J. M., LUDWIG, E. H., AND CANBY, C. M. 1950 Serological studies with microaerophilic actinomycetes. Bacteriol. Proc., p 110.
59. SNYDER, W. C., AND HANSEN, H. N. 1954 Variation and speciation in the genus *Fusarium.* Ann. N. Y. Acad. Sci., **60**, 16–23.
60. SOBIN, B. A., FINLAY, A. C., AND KANE, J. H. 1950 Terramycin and its production. U. S. Patent 2,516,080.
61. STOCKER, B. A. D. 1955 Bacteriophage and bacterial classification. J. Gen. Microbiol., **12**, 375–381.
62. TAKAHASHI, B. 1953 The isolation of a new antibiotic "flaveolin". J. Antibiotics (Japan), **6A**, 11–20.
63. THAXTER, R. 1890–91 The potato "scab". Connecticut (State) Agr. Expt. Sta. Bull., **105**, Ann. Rept., p 81–95; 153–156.
64. WAKSMAN, S. A. 1919 Cultural studies of species of *Actinomyces.* Soil Sci., **8**, 71–215.
65. WAKSMAN, S. A. 1950 *The actinomycetes. Their nature, occurrence, activities, and importance.* Chronica Botanica Co., Waltham, Mass.
66. WAKSMAN, S. A., AND CORKE, C. T. 1953 *Thermoactinomyces* Tsiklinsky, a genus of thermophilic actinomycetes. J. Bacteriol., **66**, 377–378.

67. WAKSMAN, S. A., AND CURTIS, R. E. 1916 The *Actinomyces* of the soil. Soil Sci., **1**, 99–134.

68. WAKSMAN, S. A., AND HENRICI, A. T. 1943 The nomenclature and classification of the actinomycetes. J. Bacteriol., **46**, 337–341.

69. WAKSMAN, S. A., AND LECHEVALIER, H. 1953 *Guide to the classification and identification of the actinomycetes and their antibiotics*. The Williams & Wilkins Co., Baltimore, Md.

70. WAKSMAN, S. A., REILLY, H. C., AND HARRIS, D. A. 1948 *Streptomyces griseus* (Krainsky) Waksman and Henrici. J. Bacteriol., **56**, 259–269.

71. WOLFF, M., AND ISRAEL, J. 1891 Über Reincultur des Actinomyces und seine Übertragbarkeit auf Thiere. Virchow's Arch. pathol. Anat. u. Physiol., **126**, 11–59.

72. WORK, E., AND DEWEY, D. L. 1953 The distribution of diaminopimelic acid and its relation to bacterial classification. Proc. VI Internatl. Cong. Microbiology, Rome, **1**, 50–54.

73. YOKOYAMA, Y., AND HATA, T. 1953 Serological studies on actinomycetes. II. On the chemical properties of the somatic antigens and on the hemoagglutination reaction. J. Antibiotics (Japan), **6A**, 80–86.